deriving a mature transgenic avian from said transgenic cells, wherein the tubular gland cells of the transgenic avian express the coding sequence, and the resulting protein is secreted into the oviduct lumen, so that the protein is deposited ~~onto the yolk~~ in the white of an egg; and

isolating said protein from said egg.

Claim 56 (New). An egg of claim 25, wherein said exogenous protein comprises an interferon.

Claim 57 (New). An interferon isolated from an egg of claim 35.

REMARKS

Claims 19, 21, 25, 27, 29, 33-35, and 41-57 are in this application. Claims 55-57 have been added in this response. Claims 25, 27, 35, 50, 53, and 54 have been amended. Claim 50 was rejected under U.S.C. §112, first and second paragraphs; claims 27 and 33 were rejected under 35 U.S.C. §102(b); claims 19, 21, 25, 27, 29, 34, 35, 41, 42, 44, 47, and 51-54 were rejected under U.S.C. §102(e); claims 43, 45, 46, 48, and 49 were rejected under 35 U.S.C. §103(a). Entry of the amendment, reconsideration of the rejection, and allowance of claims 19, 21, 25, 27, 29, 33-35, and 41-57 are requested.

The Amendment

The specification has been amended to correct the data in Applicants' specification on the amount of β-lactamase found in the eggs of the transgenic birds (pages 25, 36, 37, 38, and 39). Since the filing of the application on October 16, 1998, additional experiments have been performed which indicate that the amounts of β-lactamase in the eggs reported in the originally filed disclosure, although believed to be correct at the time filed, were, in fact, incorrect. The error in the data resulted from the use of a sample of β-lactamase of poor quality as a standard in the experiments. The sample had been obtained

commercially from Calbiochem, LaJolla, California (catalog no. 426205). After the filing of the application, the β-lactamase sample used in the original experiments was found to be no more than about 5% pure, instead of 100% pure as previously believed due to the product information provided by Calbiochem. (The purity of the Calbiochem lactamase was determined by comparison to a sample of 100% pure lactamase obtained from Shahriar Mobashery (Department of Chemistry, Wayne State University, Detroti, Michigan, 48202)). Because the revised numbers of the amount of β-lactamase are lower than previously reported, Applicants wish to call the discrepancy to the Examiner's attention and also to request correction of the data in the specification. The correction of inaccurate data does not constitute new matter.

The specification has also been amended to correct the data on the estimated fraction of cells in any given tissue of a G_0 bird which is believed to carry the transgene (page 34 and the insert on page 37). Again, the data as filed were believed correct at the time of filing. However, subsequent experiments have led to a change in the estimate. Because the revised estimate of the fraction of cells which are transgenic is lower than previously reported, Applicants wish to call the discrepancy to the Examiner's attention and also to request correction of the data in the specification.

Minor corrections have been made to the claims by amendment. The amended claims are supported by the application as filed.

Claim 25 has been amended to indicate that the protein which is deposited in the eggs need not necessarily be exogenous to the avian species, but may simply be exogenous to an egg of the avian species, meaning that the protein is not normally found in the avian egg, or, at least, is not normally found in that quantity. One of ordinary skill in the art, upon reading lines 5-8 of page 2, lines 20-23 of page 15, lines 5-28 of page 31, and lines 1-15 of page 32 of Applicants' specification, will readily recognize that the exogenous protein which is expressed in the oviduct and deposited in the eggs will be

foreign to the oviduct and the egg, or at least present in an unnaturally high amount, but will not necessarily be entirely foreign to the whole avian species.

Claim 27 is amended to clarify that the protein being expressed by the tubular gland cell of the transgenic avian is the protein encoded by the coding sequence. Support for this amendment can be found throughout Applicants' specification and in particular on page 8, lines 4-14, and on page 31, lines 18-22.

Claim 27 and claim 35 have also been amended to remove the limitation that the vector insertion be random. Support for these amendments can be found in statements of the specification which indicate that the integration of transgenes in the avian genome need not necessarily be random (for instance, page 31, lines 5-8; page 7, lines 17-18; page 19, lines 3-5; page 21, lines 5-6; and page 28, lines 5-7). One of ordinary skill in the art will readily recognize that although random integration of the promoter-containing expression vectors would be most common, targeted integration methods such as those analagous to those described in Applicants' specification for the promoter-less vectors (page 10, lines 5-7; page 28, lines 15-25; page 29, lines 6-9) would also be suitable.

Claim 50 has also been amended to more precisely state the claimed invention. The amendment clarifies that it is the protein encoded by the second coding sequence, not the coding sequence itself, which is capable of providing post-translational modification of the protein encoded by the first coding sequence. Support for this amendment can be found on lines 23-25 of page 23 of Applicants' specification. Hence, no new matter is added by the amendment to claim 50.

In other minor corrections, claims 53 and 54 have been amended to clarify that it is the protein encoded by the exogenous gene, not the exogenous gene itself, that is deposited into the eggs of the transgenic bird. Support for this amendment can be found throughout Applicants' specification and in particular on page 31, lines 18-22, and page 36, lines 5-9 and Table 1.

Claim 55 is new, but contains no new matter. Support for this claim to a method of producing protein can be found throughout the specification as originally filed, including page 31, lines 5-22 and claim 35. Additionally, one of ordinary skill in the art will recognize that the disclosures on page 32, lines 3-15, page 1, lines 20-28, and page 2, lines 1-13 indicate that the exogenous protein which is deposited in the eggs of the transgenic bird will be removed or isolated from the egg and put to a variety of uses where isolation from the egg would be required (for instance, as pharmaceuticals).

Similarly, claims 56 and 57 are also new, but contain no new matter. These new claims specify that the exogenous protein which is expressed in the avian oviduct and deposited in the avian eggs may comprise an interferon. Support for the new claims can be found on page 32, line 6, and in claim 41 of the originally filed application.

Entry of the amendments is respectfully requested.

Rejections Under 35 U.S.C. §112

Claim 50 was rejected under 35 U.S.C. §112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to make and/or use the invention. Claim 50 (as amended) is directed to a method of producing an avian egg which contains exogenous protein, using a vector comprising two coding sequences separated by an internal ribosome entry site element where the protein encoded by one of the coding sequences is capable of providing post-translational modification of the protein encoded by the other sequence. The Examiner asserts that the specification fails to provide sufficient guidance and fails to teach the claimed genetic construct used in the methods. More specifically, the Examiner writes that Applicants' specification "fails to provide guidance to a specific modulating enzyme for post translational modification of collagen or any other protein of interest and fails to show the claimed post translational modification of a protein"

and that "the skilled artisan at the time of filing would be lacking a reasonable expectation of success, to make an avian egg containing the post translational modified exogenous protein, without an undue amount of experimentation".

This rejection is respectfully traversed.

The Examiner's rejection of claim 50 due to lack of enablement appears to be two-fold. First, the Examiner appears to assert that because Applicants do not specifically list suitable specific combinations of proteins, their modifying enzymes, and the post-translational modifications involved, the expression vectors which would be used in the claimed method are not sufficiently taught. By way of example, Applicants, as noted by the Examiner, do specifically mention in the specification (page 23, lines 25-27) that collagen could be the protein encoded by the first coding sequence and that the second protein encoded by the vector could be a modifying enzyme which hydroxylates collagen. Still, the Examiner protests that the specification fails to provide guidance to a specific modifying enzyme capable of hydroxylation of collagen. Applicants submit, however, that no specific mention of appropriate hydroxylating enzymes is required for enablement of the invention. One of ordinary skill in the art would easily be able to identify what enzymes would be capable of catalyzing the hydroxylation of collagen. This is demonstrated in the journal article cited by the Examiner himself, Myllyla et al., *Biochem. J.,* 196:683-692, 1981 ("**Myllyla et al.**"). This article demonstrates that approximately 17 years before the filing of Applicants' specification, skilled artisans were already fully aware of at least two enzymes capable of hydroxylating collagen -- prolyl hydroxylase and lysyl hydroxylase. Undue experimentation would not be required to identify the enzymes useful for hydroxylation at the time of filing and prepare a vector accordingly; such information was readily available to one of ordinary skill in the art. Furthermore, identification of a number of alternative appropriate pairings of other specific proteins with their modifying enzymes would be feasible for one of ordinary skill in the art. Since the

courts have repeatedly held that a "patent need not teach, and preferably omits, what is well known in the art" *(Lindemann Maschinenfabrik GMBH v. American Hoist and Derrick Company et al.,* 221 USPQ 481 (Fed. Cir. 1984); *Hybritech Inc. v. Monoclonal Antibodies, Inc.,* 231 USPQ 81 (Fed. Cir. 1986)), the mention of specific, modifying enzymes should not be a requirement for enablement. Furthermore, neither the inclusion of specific examples in the specification nor the disclosure of all possible embodiments is a prerequisite for enablement (MPEP 2164.02).

Second, the Examiner appears to question whether or not the enzyme expressed by the second coding sequence on the vector would ever be able to modify the protein expressed by the first coding sequence, even if an appropriate pair of modifying enzyme and substrate were correctly identified and their coding sequences included on the vector. The Examiner states, "The state of the art at the time of filing was such that various factor affects the extent of the post translational modification of proteins." By way of example, the Examiner states that the ratio of enzyme to substrate in the cell has been suggested by **Myllyla et al.** to be a critical factor in the regulation of the post-translational modification of collagen in transformed cells. Applicants acknowledge that the precise regulation of post-translational modification of many native proteins in cells may be very complex. However, for purposes of enablement of claim 50, the complexity of regulatory mechanisms which normally control the precise post-translational modification of endogenous proteins in cells is irrelevant. The issue with respect to enablement is whether or not the expression of two proteins, one of which can, at least in some contexts, modify the other post-translationally, from a single vector in the oviduct of a bird as taught by Applicants' specification, would be reasonably expected to result in the modification of the substrate protein sometime before, during, or after the deposition of the protein in an egg laid by the avian.

Applicants submit that there would be a reasonable expectation that successful modification and deposition of the protein would be achieved. Applicants have established through specific working

examples that their procedures do result in the expression of active enzymes and deposition of the active enzymes in eggs (Examples 1-5, Applicants' specification). It is reasonable to expect that where one protein can be expressed and deposited in an egg, two can be expressed and deposited in an egg (the use of internal ribosome entry site elements for dual expression being commonplace in the art). In most cases, the two proteins on the vector would be expressed in an approximately one-to-one ratio. Since Applicants have established that the enzymes expressed in the oviduct and deposited in the eggs are active (Example 4, pages 34-37 of Applicants' specification), one of ordinary skill in the art would assume that a different enzyme which was expressed in the cells of the oviduct and which was a modifying enzyme would also be active. Therefore, it is reasonable to expect that the coexpression of a modifying enzyme in an approximately one-to-one ratio with its substrate protein (a ratio far greater than normal catalytic amounts) in the cells of an avian oviduct according to Applicants' teachings would result in the modification of at least some of the substrate protein, followed by deposition of the modified protein in the eggs of the avian. In other words, there would be a reasonable expectation of success. Thus, one of ordinary skill in the art would find Applicants' disclosure enabling with respect to claim 50.

The Examiner has not cited any art which casts doubt upon the expectation of success of Applicants' invention. However, references can be found in the art which support the feasibility of Applicants' invention by describing the successful coexpression of proteins with their corresponding modifying enzymes in cells. For instance, efficient conversion of the human protein C precursor to its mature form following coexpression of the precursor with its processing enzyme in mouse mammary glands has been reported (Drews et al., *Proc. Natl. Acad. Sci. USA*, 92:10462-10466, 1995; Paleyanda et al., *Journal of Biological Chemistry*, 272:15270-15274, 1997).

For the reasons discussed above, Applicants respectfully request withdrawal of the rejection.

Claim 50 was also rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. The Examiner asserts, "The claim fails to recite the protein encoded by first coding sequence and the enzyme required for its post translational modification encoded by the second coding sequence." While this particular assertion is true, such a failure to specifically recite a specific protein and a specific post-translational modification in a claim does not constitute a flawed claim, just a broad one. "Breadth of a claim should not be equated with indefiniteness" (MPEP 2173.04, *In re Miller*, 169 USPQ 597 (CCPA 1971)). The language of the claim is both clear and definite and one of ordinary skill in the art would readily be able to discern the scope and meaning of the claim as written (*Shatterproof Glass Corp. v. Libbey-Owens Ford Co.*, 225 USPQ 634 (Fed. Cir. 1985); *North American Vaccine, Inc. v. American Cyanamid Co.*, 28 USPQ2d 1333 (Fed. Cir. 1993)). For instance, it is understandable that the protein encoded by the second coding sequence could be any protein capable of performing any kind of post-translational modification on the protein encoded by the first coding sequence. There is no indication in the claim or anywhere else in the specification that a vector encoding a specific pair of protein and modifying protein is instead desirably claimed in claim 50, and there is no ambiguity as to what is meant by "protein" or "post-translational modification" since these are terms well-known to those skilled in the art. A rejection of claim 50 under 35 U.S.C. §112, second paragraph is unwarranted. Applicants respectfully request withdrawal of the rejection.

In light of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejections of claim 50 under 35 U.S.C. §112, first and second paragraphs.

Rejections Under 35 U.S.C. §102

Claims 27 and 33 were rejected under 35 U.S.C. §102(b) as being anticipated by Thoraval et al., *Trans. Res.*, 4:369-376, 1995 ("**Thoraval et al.**"). In the Office Action, the Examiner states that:

> Thoraval et al teaches germline transmission of exogenous genes in chickens using helper-free ecotropic avian leukosis virus based vector (see page 371, col.2, table-1 and par.1). Thus, Thoraval et al clearly anticipated the method wherein the introduction of the vector into blastodermal cell is mediated by a retrovirus.

The rejection of claims 27 and 33 is respectfully traversed.

Claim 27 is directed to a method of producing an exogenous protein in an avian oviduct by introducing a vector comprising a coding sequence operably linked to a promoter into avian embryonic cells and then deriving a mature transgenic avian from the cells, where the tubular gland cells of the mature transgenic avian express the protein. Claim 33 requires the use of retroviral-mediated delivery of the vector in the method of claim 27.

Applicants agree that **Thoraval et al.** teaches the retroviral delivery of a vector to avian blastodermal cells. However, Applicants disagree with the Examiner's assertion that **Thoraval et al.** anticipates claim 27 and 33, since **Thoraval et al.** does not disclose the generation of a mature transgenic avian which expresses the exogenous protein in the tubular gland cells of its avian oviduct. **Thoraval et al.** does disclose G_0 embryos and G_0 and G_1 hatched chicks which have the transgene present in their blood or semen (as determined by PCR analysis). It is the transgene analysis of the G_0 birds that is disclosed in the passage cited by the Examiner (**Thoraval et al.**, page 371, column 2, Table 1 and paragraph 1). **Thoraval et al.** also reports the expression of the transgenes in embryo fibroblasts derived from G_2 embryos (**Thoraval et al.**, page 373, columns 1 and 2 and Table 3). However, **Thoraval et al.** does not report the expression of the transgene in any tissue of any mature bird (or even in any young hatched bird). It cannot be assumed that simply because the transgene was present in some

or all tissues of the hatched birds that the transgene is actually being expressed in those or other tissues. The expression of a retroviral vector used as a transgene in a cell or animal may be unstable and may be shut off by such mechanisms as methylation, deletion, or mutation of the vector sequence or even may be excised from the genome. Likewise, even if cells removed from a G_2 embryo express the exogenous protein in culture, it cannot be assumed that the embryo will grow into a mature bird which still expresses the protein anywhere in its body, let alone in the tubular gland cells of its oviduct.

In order for a rejection under §102 to be valid, each and every element of the claim must be found in the prior art reference (MPEP 2131; *In re Royka and Martin*, 180 USPQ 580 (CCPA 1974)). As already described, claim 27 and claim 33 are both directed to methods for producing an exogenous protein in an avian oviduct and a required element of each method is the generation of a mature bird which expresses the exogenous protein in its oviduct. Since **Thoraval et al.** fails to teach, either expressly or inherently, any such expression or production of exogenous proteins in the oviduct of a mature bird, an anticipation rejection is improper.

In light of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejection of claims 27 and 33 under 35 U.S.C. §102(b).

Claims 19, 21, 25, 27, 29, 34, 35, 41, 42, 44, 47, and 51-54 were rejected under 35 U.S.C. §102(e) as being anticipated by the PCT publication Macarthur, WO 97/47739 (**"Macarthur"**).

This rejection is respectfully traversed.

Although Applicants disagree with the Examiner's characterization of the disclosure of **Macarthur,** Applicants' traversal does not rely on this disagreement. Rather, Applicants submit that the rejection is improper because **Macarthur** does not constitute prior art under §102(e). A reference is prior art under 35 U.S.C. §102(e) only if the reference is a U.S. patent granted on an application filed in the U.S. or which entered the U.S. under 35 U.S.C. §371 before Applicants' date of invention.

Macarthur is merely a published PCT application, not a granted U.S. patent. Therefore, a rejection under §102(e) based on **Macarthur** is improper. Applicants would also like to draw the Examiner's attention to the fact that **Macarthur** was published on December 18, 1997, two months after Applicants' claimed priority date of October 16, 1997 and thus cannot be prior art under §102(b) either. Applicants respectfully request withdrawal of the rejection of claims 19, 21, 25, 27, 29, 34, 35, 41, 42, 44, 47, and 51-54 under 35 U.S.C. §102(e).

Rejections Under 35 U.S.C. §103

Claims 43, 45, 46, 48, and 49 were rejected under 35 U.S.C. §103(a) as being unpatentable over **Thoraval et al.** in view of Kotani et al., *Hum. Gene Ther.*, 5:19-28, 1994 ("**Kotani et al.**"). With regard to this rejection, the Examiner states the following:

> Thoraval et al teaches the germline transmission of exogenous genes in chickens using helper-free ecotropic avian leukosis virus based vector (see page 371, col.2, table-1 and par.1), but does not reach the regulation of transgene expression of by the CMV promotor in ALV vector. Kotani et al teaches the use of CMV promotor in the retroviral vectors. Thus, Thoraval et al teaching the ALV vector and Kotani et al teaching the use of CMV promotor to regulate the expression of a gene, it would have been obvious to one with ordinary skill in the art to use CMV promotor to regulate the expression of a gene. One would have been also motivated to had CMV promotor in ALV vector because CMV promotor is know to provide a stronger constitutive expression.

This rejection is respectfully traversed.

Claim 43 is directed to a transgenic bird which expresses a transgene comprising a coding sequence operably linked to a cytomegalovirus (CMV) promoter in its oviduct. Claims 45 and 46 are directed to methods for producing an exogenous protein in an avian oviduct. The method of claim 45 includes the use of a vector comprising a CMV promoter. The method of claim 46 involves the use of an avian leukosis virus (ALV)-derived vector. Claims 48 and 49 are directed to methods of producing

avian eggs which contain exogenous protein. Claim 48 specifies the use of a CMV promoter, whereas claim 49 specifies the use of an ALV vector. All of the methods include the step of generating a mature transgenic bird which expresses an exogenous protein in the tubular gland cells of its oviduct. Claims 48 and 49 further require that the expressed protein be secreted into the oviduct lumen and deposited into eggs.

As discussed above, with respect to the §102(b) rejection, **Thoraval et al**. teaches the transmission of exogenous genes into chickens using an ALV-based vector. The other reference cited by the Examiner, **Kotani et al.**, teaches a variety of retroviral vectors having promoters from different sources including CMV. The disclosed use of the vectors in **Kotani et al.** is human gene therapy.

Applicants submit that the references of **Thoraval et al.** and **Kotani et al.** are not properly combined because there is no motivation in either the references themselves or in the knowledge generally available to one of ordinary skill in the art to combine them (MPEP 2143.01).

The Examiner points to no passage in either **Kotani et al.** or **Thoraval et al.** which suggests a motivation for combining the two references. Although **Kotani et al.** does disclose the use of the CMV promoter in retroviral vectors, it also discloses the use of other promoters in retroviral vectors such as SV40 and β-actin. The study disclosed in **Kotani et al.** appears to have been designed to evaluate different factors which affect retroviral vector production and transduction, not expression levels. The expression levels of the different retroviral vectors are not even reported in **Kotani et al.** for any of the disclosed vectors. Therefore, **Kotani et al.** could not have taught the superiority of vectors containing the CMV promoter for any type of expression, let alone expression in an avian oviduct. (**Kotani et al.** does not even appear to teach the superiority of the CMV promoter vectors for achieving high titer levels.) Thus, **Kotani et al.** provides no motivation for using CMV promoters in ALV vectors to achieve the expression of exogenous protein in the tubular gland cells of birds or to achieve any other

type of expression. The other reference cited by the Examiner, **Thoraval et al.**, makes no suggestion with respect to the use of alternative promoters in the retroviral vectors delivered to the birds.

The Examiner does suggest that the motivation to combine the two references would come from the general knowledge of one of ordinary skill in the art. Applicants respectfully disagree and contend that one of ordinary skill in the art would not be motivated to combine the references. Applicants agree that it is common knowledge of those skilled in the art that a CMV promoter generally provides strong, constitutive expression. However, one of ordinary skill in the art would conclude that the use of a strong, constitutive promoter such as CMV could be detrimental to the goal of generating viable transgenic birds. Integration of a transgene containing a CMV promoter could be harmful to the birds if the expression product of the transgene is at all deleterious to any of the cells of the bird. Since the promoter is strong and constitutive, the transgene could potentially be expressed in every tissue of the bird which contains the transgene. This could lead to the production of the foreign protein anywhere or everywhere in the bird. Depending on the nature of the exogenous protein, one of ordinary skill in the art would expect that the health of the bird would be detrimentally and undesirably affected. Hence, there would be little motivation for one of ordinary skill in the art to combine a CMV promoter mentioned in **Kotani et al.** with the retroviral-mediated transgenesis of **Thoraval et al.**.

Furthermore, even if the two references were properly combined, the combination would still not render the present invention obvious. All limitations of the claims must be suggested by the combination of references cited as prior art in order to establish *prima facie* obviousness (MPEP 2143.03; *In re Royka and Martin*, 180 USPQ 580 (CCPA 1974)). As outlined above, all of the rejected claims 43, 45, 46, 48, and 49 include as a limitation the requirement that the transgenic bird express the exogenous protein encoded by the transgene in its oviduct. In addition, claims 48 and 49 require that the protein be deposited into eggs laid by the bird. As already discussed above with respect to the

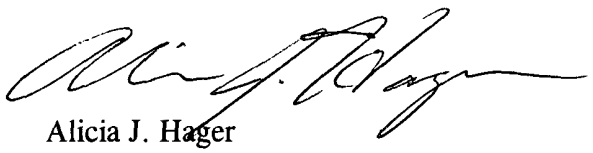
§102(b) rejection, **Thoraval et al.** fails to disclose the expression or production of an exogenous protein in the tubular gland cells of the oviduct of a mature bird. **Thoraval et al.** also fails to teach the secretion of an exogenous protein into the oviduct lumen of a mature bird followed by deposition of the protein into the eggs of the bird. The only other reference cited by the Examiner, **Kotani et al.** teaches and suggests nothing about transgenic birds. Since in each case some of the claim limitations are not suggested by the cited combination of references, the rejections are improper.

In light of the foregoing remarks, Applicants respectfully request reconsideration and withdrawal of the rejections of claims 43, 45, 46, 48, and 49 under 35 U.S.C. §103.

Conclusion

Reconsideration of claims 19, 21, 25, 27, 29, 33-35, and 41-57 in view of the foregoing remarks, and an early indication of their allowability, is earnestly solicited.

Respectfully submitted,



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